Impact of copy number variation on human neurocognitive deficits and congenital heart defects: a systematic review

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Abstract

Copy number variant (CNV) syndromes are often associated with both neurocognitive deficits (NCDs) and congenital heart defects (CHDs). Children and adults with cardiac developmental defects likely to have NCDs leading to increased risk of hospitalisation and reduce independence. To date, the association between these two phenotypes have not been explored in relation to CNV syndromes. In order to address this question, we systematically reviewed the prevalence of CHDs in a range of CNV syndromes associated with NCDs. A meta-analysis showed a relationship with the size of CNV and its association with both NCDs and CHDs, and also inheritance pattern. To our knowledge, this is the first review to establish association between NCD and CHDs in CNV patients, specifically in relation to the severity of NCD. Importantly, we found specific types of CHDs were associated with severe neurocognitive deficits. Finally, we discuss the implications of these results for patients in the clinical setting which warrants further exploration of this association in order to lead improvement in the quality of patient's life.

Abbreviations

CNV; copy number variant, NCD; neurocognitive deficit, CHD; congenital heart defect, ADHD; attention deficit hyperactivity disorder, ASD; autism spectrum disorder, ASD; atrial septal defect, VSD; ventral septal defect, PDA; Patent ductus arteriosus

Keywords

Copy number variant syndrome; neurodevelopment; neurocognitive deficit; congenital heart defect

1. Introduction

Copy number variant (CNV) syndromes are caused by rearrangements of DNA (commonly a deletion or duplication) in a section of a chromosome leading to disruption of a functional DNA segment. The amount of DNA either deleted or duplicated can vary in length, even within CNV syndromes, and is generally regarded as likely to be pathogenic if over 100kb in length[1]. Increases or decreases in the CNVs have been associated with an array of neurological, psychiatric and developmental phenotypes including intellectual disability[2], schizophrenia[3], autism[4] and ADHD[5]. The presence of environmental factors, along with CNVs can also lead to increase or decrease in severity of the disease phenotype[6]. There are currently 67 CNV syndromes associated with developmental disorders [7]. Another phenotype often associated with CNVs are heart defects[8, 9]. Genome wide association studies (GWAS) have provided evidence suggesting that CNVs are one of the significant contributors to increased risk for congenital heart disease in conjunction with neurodevelopment[10], neurodevelopmental disorders[11] and neurocognitive deficits (NCD)[12-14]. Approximately 14% of infants with single ventricle heart defects were reported to have CNVs in comparison to 4.4% of control subjects and these accompanied neurocognitive decline and retarded somatic growth[15]. Adults with congenital heart defects (CHDs) are at an increased risk of developing neuropsychiatric symptoms such as anxiety, depression, and impaired functioning involving language impairment, social cognitive decline and delayed progression to adulthood[16-18]. However, their prevalence and the likely mechanisms through which these developmental deficits may occur due to the presence of CNVs has often been limited due to the lack of longitudinal studies. Besides, there is a major effect of ascertainment bias in our knowledge of the natural history of these conditions.

The vast majority of previous research has looked at the relationship between neurodevelopment and congenital heart defects (CHDs) from the perspective of CHD cohort patients. This is to be expected as CHD appears early in life and enables prospective studies of neurodevelopment[19]. Many factors have been put forward as to why CHDs are

associated with neurodevelopmental problems. In the human fetus a large part of the heart and brain development occurs in a similar critical window and the presence of genetic alteration can impact the brain and heart development[20]. Key brain development features such as axon guidance, synapse development and cortical networks seem to be compromised in fetuses with CHDs during the third trimester of pregnancy, which can lead to disrupted neural activity[20, 21]. Studies have shown that even before corrective surgery, brain injury can occur as a result of CHDs[22], associated with impaired fetal blood flow[20]. One disputed cause is cardiac surgery in early infancy. In some cases, surgery has resulted in newly-acquired brain injury [23] and adverse neurodevelopmental outcomes[24, 25], whereas other studies have explained the differences in these outcomes by association with other variables such as demographic[26, 27] and genetic factors[28]. Brain imaging studies in school children and adolescents with CHDs show altered white matter hyperintensities, connectivity[29], which partially explains the possibility as to why we see poorer fine and gross motor function, learning difficulties and lower IQ[13, 24]. Given that CHDs are a lifelong condition, gaining insight into their association with neurodevelopmental/neurocognitive deficits is crucial as it has clinical and public health relevance in terms of diagnostic assessment and preventative strategies.

To date, there has been no studies have explored the relationship between congenital heart defects and neurodevelopmental disorders in relation to CNV syndromes. To fill this knowledge gap, we performed the systematic review to explore the relationship between neurocognitive deficit and CHDs from the perspective of CNV syndromes. Furthermore, we investigate these associations in the absence of surgery, which could be a compounding factor. Our objective of this study is to establish the prevalence of CHDs in CNVs associated with NCD and specifically what types of heart defects were associated with the cognitive deficits in the CNV patients. Given the exploratory nature of the study, we also looked at genes involved in these CNVs to explore if there can be potential converging gene pathways between the heart and cognitive defects that can explain the co-morbidity features.

2. Methods

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[30]. The protocol for this systematic review is registered on the PROSPERO database (CRD42019139036).

2.1 Search strategy

A multi-database search was performed (PubMed, EMBASE, The Cochrane Library) using the following search terms: 'neurodevelopmental delay; 'congenital heart defect'; and 'copy number variant'. Articles published in the English language between January 1st 1999 and April 30th 2019, were extracted between 20th March and 30th April 2019 **(table S1)**. Studies were extracted from the systematic literature search and selected based on their suitability, following the inclusion and exclusion criteria (**figure 1**). Inclusion criteria included the following: (i) Original CNV patient cohorts; (ii) CNV deletion or duplications; (iii) Patients with NCD and/or CHD; (iv) individual participant data is provided. Exclusion criteria included: (i) studies focusing on cardiac surgery and outcomes.

2.2 Data extraction

Individual participant data were extracted from each study. Data was either provided in the main text or supplementary materials. Participants were excluded for: additional chromosomal abnormalities; surgery before cognitive deficit could be diagnosed; patients described previously; prenatal patients; died before NCD was evident/could be tested. From all included studies, data were extracted for relevant demographics including age and gender of patients. Further, clinical data were extracted for purposes of quantitative comparison: presence of neurocognitive deficit (measured by intellectual disability, mental

delay, mental retardation, learning difficulties, developmental delay, language deficit) and severity; presence of congenital heart defect; size of CNV (Mb).

2.3 .Gene-pathway analysis

Gene-pathway analysis was undertaken to look at any potential converging pathways that implicated both the brain and heart development and functionality. To ensure a comprehensive list of genes, CNVs included in this study and additional CNVs associated with neurocognitive deficit and heart defects reported in[31] were included. Genes associated with the CNVs were acquired from the Decipher database[7] for 1p36, 1q21.1, Wolf-Hirschhorn syndrome (WHS), 5p15.2 (Cri du Chat), 7q11.23, 8p23.1, 9q, 16p13.11, 17p11.2 (Potocki-Lupski syndrome /Smith-Magenis Syndrome), 17q21.31 (Koolen de Vries syndrome), 22q11.2 and Xq28 syndromes. For 2q22-q23 (Mowat-Wilson) syndrome, the genes were acquired through NCBI RefSeq[32]. All genes were included if they were protein coding and reported in the OMIM database[33]. Genes were included in the pathway analysis if they had previously been implicated in the brain or heart (according to OMIM) or if they had biased or ubiquitous expression in the brain or heart according to NCBI RefSeq. The gene list was imported into the Reactome database[34] (version 69) and pathway analysis was performed utilising the 'analyse data' tool[35].

2.4 Data and statistical analysis

A meta-analysis was conducted on all included studies using IBM SPSS Statistics Version 25. Subgroup analysis was performed on deletion and duplications. Student's *t*-tests were performed to explore the association between CNV size and clinical variables, chi-square tests and a one-way ANOVA were used to look at the association between NCD and CHD, along with CNV size and severity of NCD. Data is presented as mean (M) and standard error of the mean (SEM). Risk of bias in individual studies was assessed using the Quality In Prognosis Studies (QUIPS) tool[33, 36] (**table S2**).

3. Results

3.1 Overview of studies included

In total, 16 studies were included in this review, looking at 12 CNV syndromes (**table 1**). All studies provided data on patients with CNVs and no control patients were looked at. All studies reported on neurocognitive deficit and CHD. The severity of NCD was reported in 10 studies. Neurodevelopmental disorders were reported by 12 studies. A total of 11 studies reported on CNV length. All except two studies reported on the gender of patients.

Neurocognitive deficits were very common among the studies of CNVs, ranging from 40% to 100%; with the majority of studies showing an incidence of neurocognitive deficits towards the upper end of that range. The incidences are shown in **figure 2**. The incidence of heart defects had a wider range from 12% to 100%. Features of the CNVs were largely homogeneous between studies with other common phenotypes including neurodevelopmental disorders such as autism and ADHD, dysmorphic facial features, brain anomalies (such as delayed myelination, hypoplasia of the corpus callosum, enlarged ventricles), skeletal anomalies, hypotonia and seizures.

The most common category of CHDs in these patients were septal defects, including VSD (26%) and ASD (24%) (**figure 3**). 22% of patients had patent ductus arteriosus (PDA), and 18% of patients had aortic narrowing (including aortic stenosis and aortic coarctation). Heart defects that occurred in only one or two patients were classed as ‘other’, which included tetralogy of Fallot, pulmonary atresia, transposition of great vessels, and Ebstein’s anomaly. All patients with PDA and ASD had cognitive deficits, and with the exception of one patient, all VSD patients also had cognitive deficits.

3.2 Meta-analysis

3.2.1 Patient Cohort

All studies were included in the meta-analysis. Inclusion was made on a patient-by-patient basis. Patients were excluded on an individual basis using the following criteria: (i) surgery was performed or patients died before neurocognitive deficit could be diagnosed, (ii) patients had additional chromosomal abnormalities, (iii) patients had been previously reported, (iv) prenatal patients. 246 out of 329 patients (74.8%) from the 17 studies were included in the meta-analysis. Demographics are shown in **table 2**.

3.2.2 CNV size

CNV size (the amount of DNA in Mb, that is either deleted or duplicated in patients) was explored to see if there was an association between length and phenotype presentation. An independent samples t-test showed patients with NCD (n = 135, M = 3.8, SEM = 0.46) had a significantly larger CNV compared to patients without a NCD (n = 8, M = 1.1, SEM = 0.20), ***$p < 0.001$. Additionally, we found a significant association for the patients with CHDs (n = 64) who had larger CNV sizes (M = 5.0, SEM = 0.73) compared to patients with no heart defects (n = 70, M = 3.1; SEM = 0.58), *$p<0.05$ (**figure 4A** and **figure 4B**, respectively). Taken together, these results suggest that the length of the CNV increases the risk for developing NCD and CHD.

We also explored CNV size and gender and found no significant difference between female (n = 85, M = 4.3, SEM = 0.63) and male (n = 63, M = 3.0, SEM = 0.55) patients, $p = 0.110$ (**figure 4C**).

To explore whether CNV length differed between CNV type, a *t*-test was performed. This showed that patients with deletions (n = 104, M = 4.8, SEM = 0.58) had on average a significantly larger CNV length compared to duplication patients (n = 56, M = 1.3, SEM = 0.07), ***$p < 0.001$ (**figure 4D**). Further, we also found that *de novo* CNVs (n = 52, M = 4.1,

SEM = 1.02) were on average larger than those transmitted from a parent (n = 38, M = 1.3, SEM = 0.09), $^{**}p<0.01$.

To explore the association between CNV size and NCD severity, a one-way ANOVA was performed, which showed that the larger the CNV size the more severe the NCD ($^{***}p < 0.001$). Patients with mild NCD had an average CNV size of 1.08Mb (n = 13, SEM = 0.18), patients with moderate NCD had a larger average CNV size of 2.64Mb (n = 19, SEM = 0.55). Patients with severe NCD had the largest CNV size (n = 33, M = 6.06, SEM = 0.70). A Tuckey's *post hoc* test showed that patients with severe NCD had significantly larger CNV sizes compared to both mild and moderate NCD patients ($^{***}p < 0.001$, and $^{**}p < 0.01$, respectively). There was no significant difference between mild and moderate patients ($p = 0.370$).

Based on this finding, a sub-group analysis was performed looking at CNV size and clinical phenotypes separately in deletion and duplication patients. In deletion patients, CNV size was larger for both (NCD) (4.9 vs. 0.3) and CHD (5.7 vs. 4.1) patients, however this did not reach statistical significance ($p = 0.288$ and $p = 0.186$, respectively). Similarly, there was no difference between groups for duplication patients.

3.2.3 Association between NCD and CHD

To explore if there was a relationship between neurocognitive deficits and congenital heart defects a chi-square test was performed. The results showed no significant association between patients who had a NCD and patients who had a CHD ($p = 0.096$). Due to the small number of participants who did not have a NCD (n = 8), this relationship was further explored in relation to the number of CHDs. An independent samples *t*-test showed a significant difference in number of congenital heart defects and neurocognitive deficit. Patients with NCD were more likely to also have heart defects (M = 0.69, SD = 0.96) compared to those without NCD (M = 0.33, SD = 0.49) (**figure 5A**). Further, a one-way ANOVA showed a significant difference between the number of CHDs and severity of NCD

(*p < 0.05). A Tukey's *post hoc* test showed patients with severe NCD (n = 42, M = 0.9) were more likely to have numerous CHDs compared to patients with moderate NCD (n = 31, M = 0.4) (p = 0.027) (**figure 5B)**. No association was found for patients with mild NCD.

For the most common CHDs a chi-square was performed to look at the association between NCD severity and type of CHD (**table 3**). Overall heart defects and functional heart problems were not associated with severity. However, both PDA and VSDs were significantly associated with the severity of NCD with 67% and 80% of patients with PDA and VSD, respectively, having severe cognitive deficits. ASDs were not associated with severity of NCDs.

3.3 Gene-Pathway analysis

Another aim of the current study was to investigate if the CNVs involved in NCD and CHDs converge on common pathways that could a factor of these co-morbid symptoms. For this, a total of 126 genes from the 13 CNVs were analysed to establish the gene pathways or gene networks. In total, 958 pathways were found. Pathways of interest, which are of significance to the current study, were explored further: (i) cardiac conduction; (ii) neuronal system; and (iii) developmental biology. The cardiac conduction pathway analysis resulted in the least number CNV of genes (n = 6),followed by neuronal system that involved a slightly higher number (n = 8) and the developmental biology pathway which had the largest number of genes (n = 16), most of which were implicated in axon guidance (n = 12). Our analysis did not result in any single converging pathway that is of significant importance to the two different clinical phenotypes being studied. Genes and their corresponding CNVs and pathways is presented in **figure S1** and are described in **tables S3** and **S4.**

4. Discussion

To our knowledge, this is the first meta-analysis, based on a systematic review, to establish an association between NCDs and CHDs; whose subjects have CNVs in common. In this review we found that length of copy number variation was associated with neurocognitive deficits, congenital heart defects, inheritance of CNV and type of CNV (deletion or duplication). We also found that gender was not associated with CNV size. Further, we showed the number of heart defects a patient had were associated with increased severity of neurocognitive deficit, and PDA and VSD were associated with severe cognitive deficit.

4.1 CNV size

A larger CNV size would generally encompass an increased number of genes, and one could surmise these CNVs would therefore be associated with more symptoms, and/or an increased number of genes associated with a phenotype and thus enhancing the severity. This concept is supported by [37] who showed that more genes; and larger copy number lengths deleted are associated with an increased risk of neurodevelopmental disorder (autism) and lower IQ. A CNV that is larger than 500Kb is present in only 8% of the population[1]. From previous studies it can be concluded that larger CNVs are benign in nature[38, 39] and small CNVs show increased risk towards clinically important disorders[40].

In concordance with other studies, we found that twice as many participants had deletions compared to duplications, which relates to wider population studies suggesting that deletions occur twice as much as duplications[41], although in the general population duplications are more common[42]. We found that the deletions identified in the patients reported were on average almost four times as large as in the duplications found; duplications encompassed around 1Mb whereas deletions averaged over 4.5Mb. One possible explanation for this is the mechanism through which deletions and duplications can occur. It is generally accepted

that both involve non-allelic homologous recombination (NAHR)[43]. It has been suggested that deletions are caused by intrachromatidal NAHR; whereas duplications and deletions are caused by interchromosomal NAHR[44] or nonhomologous end-joining (NHEJ) mechanisms[45]. However, other studies have shown contrasting results to ours, finding lower copy numbers in deletions compared to duplications[46]. Furthermore, this interpretation suggests that duplications would be more likely to be ascertained through their clinical effects than deletions of the same chromosomal region, which is contrary to general clinical experience.

We found no difference in copy number between males and females in this analysis. Larger CNVs associated with neurodevelopmental disorders have sometimes been reported in females[47]. In previous studies, sex differences have been found in specific CNVs, and some have found that males were slightly more likely than girls to have ID (1.6:1)[48]. However, there was a great variation of male-to-female ratio depending on the genomic location of the CNV, with more females diagnosed with 22q13 and more males diagnosed with 22q11.2. On the other hand, whilst autism is more commonly found in males in the general population [49, 50], females are often more likely than males to have autism in CNV syndromes; particularly if occurrence is *de novo* [51, 52]. Further, some have shown no difference between the occurrence of CNVs between males and females across a range of CNV sizes[53] and no difference in size of *de novo* CNVs between males and females with autism spectrum disorders [54].

4.2 Association of CHDs and neurocognitive deficits

We found that 98% of patients with the three most common heart defects (ASD, VSD and PDA) also had neurocognitive deficits. Of the patients with CHD data, 94% had neurocognitive deficits and therefore we were not able to find a significant or meaningful result of the relationship of neurocognitive deficit and congenital heart defect presence. One problem these studies have is that there does not seem to be a common consensus for how

to assess neurocognitive deficits. The studies used a range of terms including mental retardation, intellectual disability, learning disability, developmental delay etc. Further, some studies used formal IQ testing, whereas some did not, and other studies did not state the methods used. Due to the limited research of these two variables in CNVs, we were unable to select for this during screening. Nonetheless, we did show that the number of heart defects a patient had were associated with increased severity of neurocognitive deficit. Further, we found that both VSDs and PDA were associated with more severe neurocognitive deficits. Compared to other CHDs including TOF, VSDs have been associated with developmental abnormalities[55]. Current research on this association is disputed, with some finding no association between PDA and neurodevelopment[56], whereas others have suggested a link[57]. Overall, these findings suggest a relationship between neurocognitive deficits and congenital heart defects.

4.3 Genotype-phenotype relationship

A gene-pathway analysis found several genes associated with multiple pathways. We focused on pathways associated with cardiac conduction, the neuronal system and development, which is the focus of the current study. Of particular interest was CACNB4 gene (L-type calcium voltage-gated channel auxiliary subunit beta 4) which emerged as the only gene that was common among the three pathways. This calcium-channel subunit gene is particularly associated with epilepsy in infancy[58]. Additionally, defects in this gene associated ataxia through dysfunction in Purkinje cells within the cerebellum[59]. CACNB4 is important for cardiac function and is associated with cardiac muscle contraction. A recent genome-wide association study (GWAS) of an African-American population has identified that this gene is involved in idiopathic cardiomyopathy[60]. Some studies have reported no expression of this gene in rodent heart tissue[61]. Contrastingly, other research has suggested genes of the L-type calcium channel (of which CACN4B is a member) are associated with the heart, particularly cardiogenesis and cardiac contraction[62]. Further, animal studies have shown expression during development in the heart[63, 64]. The reason

for this disparity could be because the CACNB4 gene produces at least four different transcripts due to alternative splicing[65]. Based on the function of this gene, it is plausible to hypothesise that Ca^{2+} transport/Ca^{2+} mediated signaling dysfunction are very likely mechanisms underlying CNV mediated NCD and CHD, and further studies are necessary to establish this association.

We were not able to perform a sufficient gene-pathway analysis due to the number of genes that have not yet been implicated in both heart and brain phenotypes; further, current pathways only implicate a couple of genes (e.g. LIMK1 and MYH11 in signalling by Rho GTPases). **Table 4** reviews the current research on the roles CNV genes and their potential implications in neurodevelopmental and congenital heart defects. CNVs involve a dosage effect whereby genes differ in expression depending on the type of CNV (deletion or duplication)[66-68] and there is a general difference in expression levels in the brain and heart. Further, genes may play different roles in both the central nervous system and the heart. More research needs to be conducted on genes associated with disease, in regards to neurological and cardiac phenotypes to allow analysis of converging pathways in CNVs that implicate both the brain and heart. This would then enable improved disease management and treatment which can be optimised and targeted as a whole rather than two separate clinical manifestations.

4.4 Strengths and limitations

The results of our systematic review/meta-analysis should be considered in the light of its strengths and limitations. As for the strengths, we pre-registered the protocol in a publicly available repository (PROSPERO), as to reduce the risk of multiple reporting. Further, we were able to establish the association between neurocognitive deficits and congenital heart defects among patients with CNVs. Additionally, we were able to show CNV size is implicated in both of these phenotypes and strongly suggests gene-dosage effect. There is

limited research conducted on this association in CNVs; nonetheless, we were able to identify the gaps/disadvantages in patient care which has not been reported before.

We could not assess direct association between presence of NCD and CHD due to the high number of patients with NCD. There is a possibility of variability in the quality of studies included in the current meta-analysis. Nevertheless, this did mean we could accurately assess the prevalence of CHDs in patients with NCD. Our results may not be valid for the whole CNV population because most studies were conducted with a selective and sometimes limited number population. However, a number the most common CNVs[69] were included (7q11.23, 17q21.31, 22q11.2). We could not find any converging gene pathways that were comorbid with both clinical presentations. Firstly, because each CNV could have very distinct pathways. Secondly, gene expression is more commonly reported in adulthood, which makes it challenging to determine its role during early development in the heart and brain. Thirdly, it is likely that CNV deletion and duplication of multiple genes activate or deactivate a specific subset of novel genes that contributes towards pathogenesis.

Large multicenter studies are needed to determine the associations between neurocognitive deficits and CHDs which can also be used to establish the quality-of-life in patients.

4.5 Clinical implications

In this review, we have shown that congenital heart defects and neurocognitive defects are comorbid disease phenotypes in CNVs; and this knowledge has important implications for management of these syndromes. From the studies included, patients were generally referred for cytogenetic testing for neurological defects. Patients are also commonly referred for complex congenital heart defects. After testing; often by FISH or CGH, patients are diagnosed, for example, with the 22q CNV, and undergo disease phenotyping; commonly by the referring clinician.

Perinatal counselling for congenital heart disease is generally determined by the success of surgical intervention and secondarily by the presence of genetic association. If perinatologists or fetal cardiologists are unaware of the implications of CNV in a given cardiac pathology, maternal choice on the continuation of pregnancy would not be an informed decision owing to incomplete counselling[70, 71]. Children with neurocognitive deficits are expected to be cared for by community paediatricians, rather than neurologists, who would also coordinate the care of affected children between different specialists. In reality, such a coordination with a shared care arrangement is less likely to occur owing to lack of knowledge of CNVs and its influence on the whole spectrum of human physiological systems. Similarly, the association of CNVs and delayed development may not be recognised in some cases of congenital heart diseases by cardiologists and cardiac surgeons prior to undertaking bypass surgery which renders their prognostic estimation of surgical intervention incorrect[72]. Furthermore, it is possible patients are not getting the wholistic treatment they need. Thus, there is a pressing need to increase awareness among clinicians of the importance of CNVs and their influence on the aetiology and neurological outcomes of congenital heart defects[31].

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6. Conflict of interest

The authors declare no conflict of interest.

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8. Figure legends

figure 1. Flow diagram showing the study selection and exclusion process according to PRISMA guidelines.

figure 2. Forest plot showing incidence of neurocognitive deficit. A fixed-effects model with freeman double arcsine proportion showing the incidence of neurocognitive deficits in the studies included in the meta-analysis.

figure 3. Pie chart showing prevalence of CHD type. The most common heart defects were septal defects followed by PDA in the studies included in the review.

figure 4. **The association of the CNV size on the neurocognitive deficits and congenital heart defects.** A) Bar charts showing a significant difference between patients with and without NCD. B) Patients with CHD have larger CNV sizes compared to patients without CHD. C) Duplication patients have a significantly larger CNV size compared to duplication patients. D) Females and males do not have significantly different CNV lengths on average. Error bars represent SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

figure 5. Bar graphs showing the association of average number of congenital heart defects and severity of neurocognitive deficit. A) In CHD patients, increasing number of congenital heart defects is associated with severity of neurocognitive deficit. B) A one-way ANOVA looking at severity of neurocognitive deficit and average number of CHDs. Error bars represents SEM. * $p < 0.05$.

9. Tables

Table 1. Overview of studies included in review

CNV syndrome	Patients (n)	NCD [a] (%)	CHD (%)	Other clinical symptoms	Ref.	Genes associated with neurodevelopment or heart development
1p36	33	94	67	Poor social interaction, self-injury, microcephaly, brain abnormalities including myelination delay and enlarged ventricles, craniofacial features, skeletal defects, urogenital abnormalities, hypotonia, epilepsy, eye problems, hearing problems	[73]	*CASZ1, GABRD, GNB1, KCNAB2, KLHL17, MTHFR, PLCH2, PRDM16, RERE, RNF207, SKI, SLC45A1*
1q21.1	34	82	27	Autism, ADHD, aggressive behaviour, microcephaly, craniofacial features, skeletal defects, hypotonia, seizures, cataracts	[74]	*BCL9, GJA5, NOTCH2NL, PRKAB2*
Wolf-Hirschhorn syndrome	7	86	57	Craniofacial features, growth delay, hypotonia, epilepsy	[75]	*FGFRL1, WHSC1*
	13	100	31	Microcephaly, craniofacial features, growth delay, urogenital abnormalities, hypotonia, seizures, eye anomalies	[76]	
7q11.23	14	86	29	ADHD, autism, autistic features, brain anomalies including enlarged ventricles, hypotonia, epilepsy	[77]	*CLIP2, DNAJC30, ELN, GTF2I, LIMK1*
Williams-Beuren syndrome	10	90	100	Facial dysmorphisms, connective tissue abnormality, hypocalcaemia	[78]	
	5	40	80	Impaired social interaction, dysmorphic facial features, epilepsy	[79]	
9qter	8	100	25	Behavioural problems including hyperactivity, microcephaly, brain anomalies, craniofacial features, hypotonia, seizures, infections, eye problems	[80]	*EHMT1, ZNF589*
15q13	17	94	12	Poor attention, aggressive behaviour, autistic features, brain anomalies including enlarged ventricles, craniofacial features, skeletal features, hypotonia, eye problems (strabismus)	[81]	*CHRNA7, OTUD7A*
16p13.11	33	61	12	ASD, craniofacial features, brain anomalies including delayed myelination, hypotonia, seizures	[82]	*MYH11, NDE1, NOMO1, NOMO3*
17q21.31	9	100	33	Hypotonia, dysmorphic facial features, epilepsy, skeletal deformities, urologic anomalies, brain anomalies	[83]	*CRHR1, KANSL1, MAPT*
	27	85	33	Behavioural problems, brain anomalies including enlarged ventricles, craniofacial features, growth deficiency, skeletal anomalies, renal anomalies, hypotonia, seizures, hearing impairment	[84]	
	11	100	55	Brain anomalies, facial dysmorphisms, skeletal anomalies, urological defects,	[85]	

				hypotonia, seizures, hearing loss		
17q23.1q23.2	7	100	71	Behaviour abnormalities, microcephaly, dysmorphic facial features, skeletal anomalies, hearing loss	[86]	*TBX4*
22q11.2	11	64	73	Dysmorphic facial features, recurrent infections, skeletal abnormalities, renal abnormalities	[87]	*ARVCF, CLTCL1, COMT, CRKL, DGCR6L, DGCR8, GSC2, LZTR1, PI4KA, RTN4R, SEPT5, SLC25A1, TANGO2, TBX1, TXNRD2, UFD1L*
Xq28	13	100	15	Autistic features, brain anomalies, craniofacial features, gastrointestinal problems, infections, hypotonia, seizures	[88]	*GDI1, RPL10, TAZ*

[a]NCD includes mental retardation/delay, intellectual/learning disability, developmental delay and language delay

CNV; copy number variant, NCD; neurocognitive deficit, CHD; congenital heart defect, ADHD; attention deficit hyperactivity disorder, ASD; autism spectrum disorder

Table 2. Demographics of participants included in the meta-analysis

Demographics	**% (n)**
CNV syndrome	
1q21.1	13.5 (34)
1p36	13.1 (33)
Wolf-Hirschhorn syndrome	7.9 (20)
7q11.21	5.6 (14)
WBS	6.0 (15)
9qtel	3.2 (8)
15q13	6.7 (17)
16p13.11	13.1 (33)
17q21.31	18.7 (47)
22q11.2	2.8 (11)
Xq28	5.2 (13)
Type of CNV	
Deletion	71.8 (181)
Duplication	28.2 (71)
CNV Inheritance pattern	
De novo	29.4 (74)
Inherited	24.6 (62)
Unknown	46.0 (116)
Size of deletion (mb), *mean (SD) / range*	3.55 (5.07) / 0.15 – 31.00
Age, *mean (SD) / range*	6.50 (6.39) / 0-44
Gender	
Female	44.0 (111)
Male	37.7 (95)
Unknown	18.3 (46)
Neurocognitive deficit	
Yes	85.3 (216)
No	6.0 (15)
Unknown	8.7 (22)
Neurodevelopmental disorder	
Yes	19.8 (55)
No	37.3 (94)
Unknown	42.9 (108)
Congenital heart defect	
Yes	38.9 (98)
No	48.0 (121)
Unknown	13.1 (33)

Table 3. Association of severity of neurocognitive deficit and types of CHD

	NCD, % (n)			X^2	*p*
CHD type	Mild	Moderate	Severe		
CHD	37 (11)	28 (9)	52 (22)	4.681	0.096
PDA	7 (1)	27 (4)	67 (10)	6.083	**0.048**
ASD	25 (3)	33 (4)	42 (5)	0.104	0.949
VSD	13 (2)	7 (1)	80 (12)	11.583	**0.003**
Functional	0 (0)	50 (3)	50 (3)	2.748	0.253

NCD; neurocognitive deficit, CHD; congenital heart defect, PDA; patent ductus arteriosus, ASD; atrial septal defect, VSD; ventral septal defect

Table 4. Potential role of CNV genes in neurodevelopmental and heart defects

CNV syndrome	Gene	Pathway	Gene expression[a] (TPM)		Potential role
			Brain	Heart	
1p36	*CASZ1*	N/A	0.7	5.7	This gene shows that significant problems/defects can occur even at low expression. CASZ1 had been implicated in both brain and heart development, with animal models showing knockouts of this gene cause reduced proliferation of cardiomyocytes and ventral septal defect, and is implicated in neurogenesis[89].
	RERE	Apoptosis	26.6	39.8	RERE has been implicated in many organs[90] and is highly expressed in the brain and heart. RERE has been implicated in many roles including cerebellar development[91], and heart tube looping[92].
	SKI	Signal transduction > signalling by TGF-beta > BMP / transcriptional activity of SMAD2/SMAD3:SMAD4 heterotrimer	28.7	10.4	SKI is highly expressed in the ascending aorta during development and is thought necessary for efficient regulation of TGF-β signalling during cardiac development[93]. Further, SKI knockdown has been associated with reduced proliferation of active astrocytes, potentially through the Ras-Raf-ERK1/2 signalling pathway[94].
1q21.1	*GJA5*	Vesicle-mediated transport > gap junction trafficking and regulation	1.7	23.2	GJA5, also known as connexin 40, is a gap junction protein associated with tetralogy of Fallot[95] and atrial fibrillation[96]. Gap junctions have long been associated with both brain[97] and heart[98].
WHS	*WHSC1*	DNA repair > DNA double-strand break response Chromatin organisation	35.5	10.5	Mice with reduced levels of this gene have congenital heart defects; including septal defects[99]. WHSC1 has been implicated in DNA damage and repair[100, 101].
7q11.23 / Williams Syndrome	*LIMK1*	Signal transduction > RHO GTPases activate PAKs/ROCKs Axon guidance > semaphoring interactions/EPH-Ephrin signalling	39.5	4.1	LIMK1 is part of a pathway that signals through PAKs/ROCK, of which 16p13.11 associated gene (MYH11) is also a member. Whilst more highly expressed in the brain, LIMK1 has been implicated in both the brain and heart. Increased expression of this gene has been associated with atrial fibrillation[102] and impaired neuronal migration[103].
9qter	*EHMT1*	Cellular response to external stimuli Chromatin organisation Gene expression > transcriptional	19.6	8.9	Haploinsufficiency of this gene is the major cause of Kleefstra syndrome and 9q34 deletion syndrome[104] and is associated with both severe mental retardation and cardiac anomalies[105].

		regulation by E2F6			
16p13.11	*MYH11*	Signal transduction > RHO GTPases activate PAKs/ROCKs/CIT Axon guidance > semaphoring interactions/EPH-Ephrin signalling Smooth muscle contraction	5.6	23.5	MYH11 is a smooth muscle gene. Smooth muscle cells have been shown to be essential for the formation of the ductus arteriosus during development[106]. Defects in this gene have been associated with been associated with patent ductus arteriosus[107] and thoracic aortic dissection[108].
17q21.31	*KANSL 1*	Chromatin organisation	10.2	3.4	A heterozygous mutation in KANSL1 is sufficient to cause Koolen-De Vries syndrome; associated with intellectual disability and heart defects[109].
17q23.1q23.2	*TBX2*	N/A	2.6	4.4	TBX2 encodes a transcription factor which has many roles in early development including the heart and brain. This gene is particularly implicated in the development of the atrioventricular canal[110, 111] and the early anterior forebrain region[112].
22q11.2	*CLTCL 1*	Axon guidance > semaphoring interactions/EPH-Ephrin signalling Vesicle-mediated transport > gap junction trafficking and regulation	3	5.5	CLTCL1 encodes the protein CHC22 which has been shown to be upregulated in the brain during development[113]. This gene has been implicated in muscle repair[114], however a cardiac function has yet to be discovered.
Xq28	*RPL10*	Metabolism of proteins > eukaryotic translation initiation/elongation Metabolism of RNA/amino acids Axon guidance > signalling by ROBO receptors	989	1561.9	RPL10 encodes a ribosomal protein that has been largely implicated in intellectual disability[115, 116] and has also been associated with pulmonary stenosis[117].

[a]According to The Human Protein Atlas[118]

TPM; transcript per million

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11. Supplementary materials

Table S1. Example of systematic search

Database	Concept	Concept input	Combined with filters
Pub med	1	((((((((((Congenital heart disease*) OR Congenital heart defect*) OR Congenital heart anomal*) OR Congenital heart disorder*) OR Heart disease*) OR Heart defect*) OR Heart anomal*) OR Heart disorder*) OR Cardiac*) OR Cardiac defect*) OR Cardiac anomal*) OR Cardiac disease*) OR Cardiac disorder*	Publication dates: From 1999/01/01 to 2019/04/30 Languages: English
	2	(((Neurodevelopmental disorder*) OR Neurodevelopmental delay*) OR Developmental delay*) OR Intellectual disabilit*	
	3	Search ((((((CNV*) OR Copy number variant*) OR Chromosom*) OR Chromosom* defect*) OR Chromosom* anomal*) OR Chromosom* disorder*) OR Chromosom* abnormal*	
EMBASE	1	All key words in concept 1 entered in separately and selected OR for all	Publication year: from 1999 to current Language: English Article type: do not include conference abstracts
	2	All key words in concept 2 entered in separately and selected OR for all	
	3	All key words in concept 3 entered in separately and selected OR for all	
Cochrane Library	1	(Congenital heart disease*):ti,ab,kw OR (Congenital heart defect*):ti,ab,kw OR (Congenital heart anomal*):ti,ab,kw OR (Congenital heart disorder*):ti,ab,kw OR (heart disease*):ti,ab,kw OR (heart defect*):ti,ab,kw OR (heart anomal*):ti,ab,kw OR (Heart disorder*):ti,ab,kw OR (Cardiac*):ti,ab,kw OR (Cardiac defect*):ti,ab,kw OR (Cardiac anomal*):ti,ab,kw OR (Cardiac disease*):ti,ab,kw OR (Cardiac disorder*):ti,ab,kw	From 1999 to 2019 All articles in the English language
	2	(Neurodevelopmental disorder*):ti,ab,kw OR (Neurodevelopmental delay*):ti,ab,kw OR (Developmental delay*):ti,ab,kw OR (Intellectual disabilit*):ti,ab,kw	
	3	(CNV*):ti,ab,kw OR (Copy number variant*):ti,ab,kw OR (Chromosom*):ti,ab,kw OR (Chromosom* defect*):ti,ab,kw OR (Chromosom* anomal*):ti,ab,kw OR (Chromosom* disorder*):ti,ab,kw OR (Chromosom* abnormal*):ti,ab,kw	

Table S2. Table showing bias of each study based on the QUIPS tool

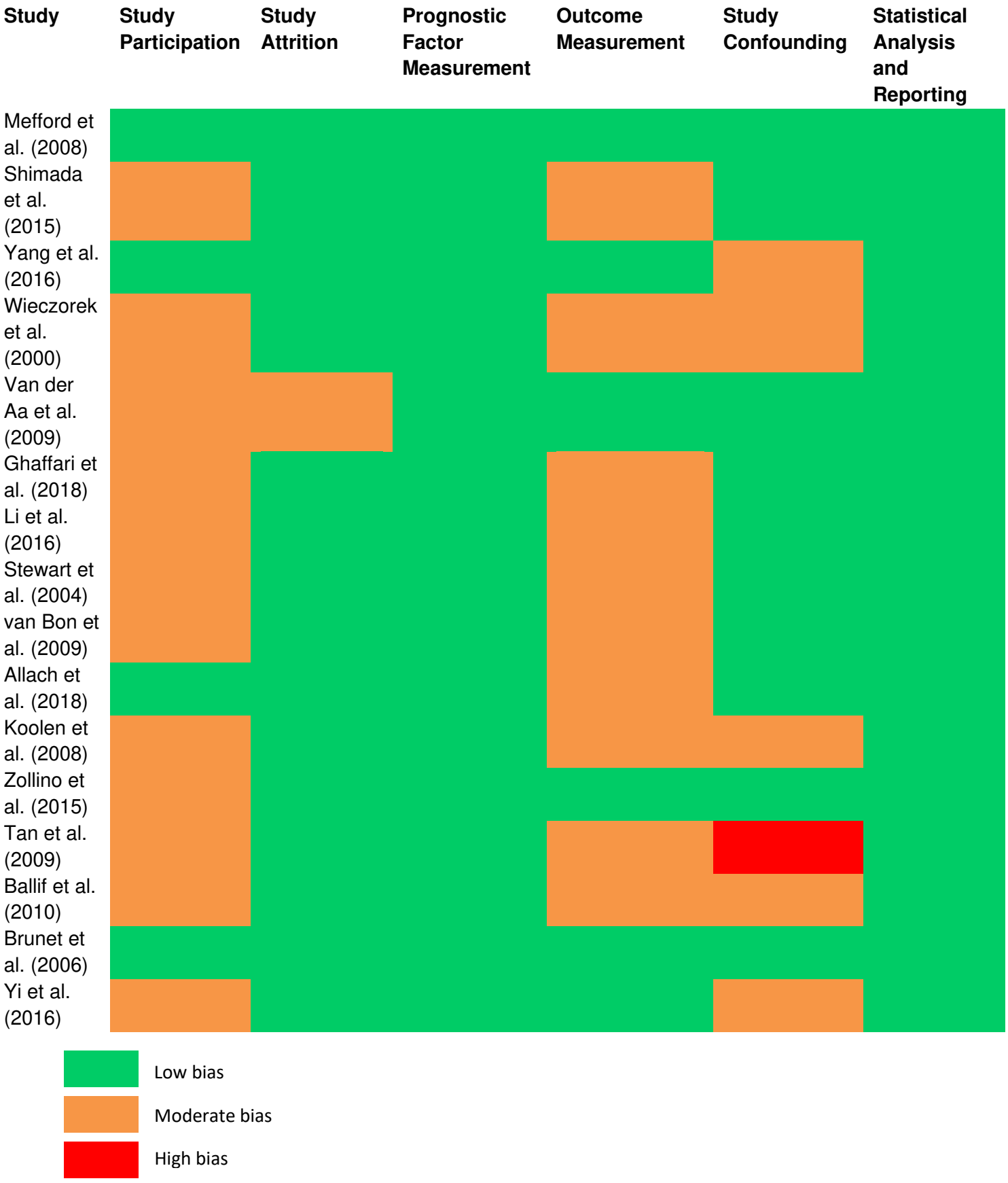

Table S3. Genes involved in cardiac, neuronal and developmental pathways

Pathway	Genes	CNV
Cardiac conduction		
YAP1- and WWTR1 (TAZ)-stimulated gene expression	*NPPA* *GATA4*	1p36 8p23.1
LTCC heteropentamer (open) transports Ca2+ from extracellular region to cytosol	*CACNB4*	2q22-q23
Signalling by Hippo Transcriptional activity of SMAD2/SMAD3:SMAD4 heterotrimer	*TAZ*	Xq28
L1CAM interactions	*SCN1A*	2q22-q23
Unknown	*NPPB*	1p36
Neuronal system		
Potassium channels	*GNB1*	1p36
Transmission across chemical synapses	*GNB1* *CACNB4* *COMT* *PRKAB2*	1p36 2q22-q23 22q11.2 1q21.1
Neurotransmitter clearance	*COMT* *SLC6A3*	22q11.2 5p15.2
Neurotransmitter receptors and postsynaptic signal transmission	*PRKAB2* *ADCY2* *SLC6A3*	1q21.1 5p15.2 5p15.2
Neurotransmitter uptake and metabolism in glial cells	*MAPT*	17q21.31
Neurotransmitter release cycle	*STX1A*	7q11.23
Developmental biology		
Axon guidance	*CACNB4* *MAPT* *SCN1A* *PLXNA3* *MYH11* *RPL10* *LIMK1* *MAPK7* *CLTCL1* *SEMA5A* *AGRN* *SCN1A*	2q22-q23 17q21.31 2q22-q23 Xq28 16p13.11 Xq28 7q11.23 17p11.2 22q11.2 5p15.2 1p36 2q22-q23
NCAM signalling for neurite outgrowth	*CACNB4*	2q22-q23
Activation of HOX genes during differentiation	*PRKAB2* *GATA4*	1q21.1 8p23.1
Transcriptional regulation of pluripotent stem	*PRKAB2*	1q21.1

cells		
Regulation of beta-cell development	*MAPT* *GATA4*	17q21.31 8p23.1
LGI-ADAM interactions	*STX1A*	7q11.23
Transcriptional regulation of adipocyte differentiation	*STX1A* *GATA4* *MED9*	7q11.23 8p23.1 17p11.2
Transcriptional regulation of granulopoiesis	*GATA4* *TAZ*	8p23.1 Xq28
Myogenesis	*TAZ*	Xq28

Table S4. Brain and heart expression of genes involved in cardiac, neuronal and developmental pathways

Genes	Pathway	Gene expression (TPM)	
		Brain (Cerebral cortex)	Heart
GNB1	Neuronal system	447.4	79.5
COMT	Neuronal system	109.8	51.3
SLC6A3	Neuronal system	0	0
ADCY2	Neuronal system	57.2	0
NPPA	Cardiac conduction	16.9	24003.4
NPPB	Cardiac conduction	0	3255.2
PLXNA3	Developmental biology (axon guidance)	4.1	1.9
MYH11	Developmental biology (axon guidance)	5.6	23.5
RPL10	Developmental biology (axon guidance)	989	1561.9
LIMK1	Developmental biology (axon guidance)	39.5	4.1
MAPK7	Developmental biology (axon guidance)	3.8	2.1
CLTCL1	Developmental biology (axon guidance)	3	5.5
SEMA5A	Developmental biology (axon guidance)	14.5	6.1
AGRN	Developmental biology (axon guidance)	33.2	3.5
MED9	Developmental biology	17.9	14.8
PRKAB2	Neuronal system Developmental biology	22.6	16.6
STX1A	Neuronal system Developmental biology	166.6	0.5
MAPT	Neuronal system Developmental biology (axon guidance)	116.2	2.5
TAZ	Cardiac conduction Developmental biology	7.1	13.3
GATA4	Cardiac conduction Developmental biology (axon guidance)	0	59
SCN1A	Cardiac conduction Developmental biology (axon guidance)	10.3	0

CACNB4	Cardiac conduction Neuronal system Developmental biology (axon guidance)	25.9	0.1

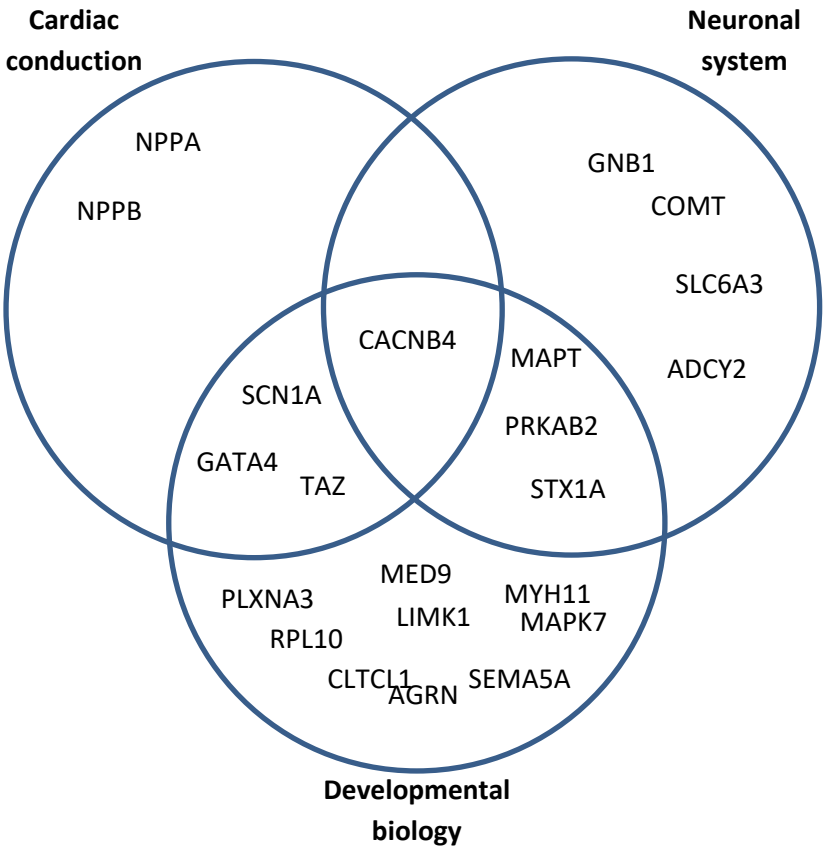

Figure S1. Venn diagram showing overlap of genes in pathways. Many genes from the CNVs are associated with two or more pathways involved in neuronal and cardiac development/function. CACNB4 is associated with all three pathways.